REMARKS

After amendment, claims 25-28, 31, 34, 40-45 and 47-58 remain pending in the present application, claims 25-28, 31, 34 and 52-58 being currently amended and claims 40-44 and 47-51 having been previously amended, claim 45 being an original claim. Support for the amendment to the claims can be found in the original specification and claims. No new matter has been added by way of the present amendment. Applicants respectfully request that these amended claims be considered in determining the allowability of the present application.

The Examiner has variously objected to or rejected the previously filed amended claims and specification for having failed to file a sequence listing, for having failed to provide a table 4, and under 35 U.S.C. §§112, first and second paragraphs, §§102(b) and 103, and under the judicially created doctrine of obviousness-type double patenting. Applicants shall address each of the objections/rejections in the sections which follow herein.

The Filing of a Sequence Listing

The Examiner objected to the originally filed application as having failed to provide a sequence listing. Enclosed please find the sequence listing, and an electronic copy of same in compliance with the requirements of 37 C.F.R. §1.821-1.825. The specification has been amended accordingly.

The Objection to the Disclosure

The Examiner has objected to the disclosure of the original application for the reasons set forth in the office action on page 3 in paragraph 3. In response, Applicants have deleted line 34 on page 6 of the specification.

The §112, Second Paragraph Rejection

The Examiner has rejected claims 26, 27 and 53-58 for the reasons which are stated in the office action on page 3 in paragraphs 5-6. With respect to the Examiner's rejections, Applicants provide the following:

On page 3, in paragraph 6A of the office action, with respect to the Examiner's rejection that claim 26 is ambiguous in the recitation of "6-19", line 5 as a surface market specific for mesenchymal precursor cells, Applicants respond that the recitation of the marker "6-19" would be clearly understood by a person skilled in the art. As support, the attention of the Examiner is drawn to the following documents, copies of which are enclosed for the Examiner's review.

Duerst RE, Rose D, Frantz CN.
Complement depletion in vitro limits monoclonal antibody 6-19-dependent complement-mediated killing of tumor cells in bone marrow.
Exp Hematol. 1991 Oct;19(9):863-7.

Iyer J, Duerst RE, Looney JN, Humphries RK, Abboud CN, Frantz CN.
An 80,000-kd glycoprotein cell surface antigen found only on nonhematopoietic cells in human bone marrow.
Exp Hematol. 1990 Jun;18(5):384-9.

Abboud CN, Duerst RE, Frantz CN, Ryan DH, Liesveld JL, Brennan JK.
Lysis of human fibroblast colony-forming cells and endothelial cells by monoclonal antibody (6-19) and complement.
Blood. 1986 Dec;68(6):1196-200.

Duerst RE, Ryan DH, Frantz CN.
Variables affecting the killing of cultured human neuroblastoma cells with monoclonal antibody and complement.
Cancer Res. 1986 Jul;46(7):3420-5.

Frantz CN, Duerst RE, Ryan DH, Constine LS, Gelsomino N, Rust L, Gregory P.
A monoclonal anti-neuroblastoma antibody that discriminates between human nonhematopoietic and hematopoietic cell types.
Hybridoma. 1986 Winter;5(4):297-306.

On page 3, in paragraph 6B of the office action, the Examiner has rejected claims 26, 27 and 53-57 as being in being indefinite and ambiguous for the reasons which are cited in the office action on page 3, paragraph 6B. In order to address the Examiner's rejection, amendments have been made to claims 26, 27 and 53-58 to refer to the cell surface molecules such as STRO-1, VCAM-1, THY-1 and CD146 as "markers". A person skilled in the art would understand that it is common in this field of technology for a cell surface molecule and an antibody that reacts with the cell surface molecule to be given the same name. This is consistent with the way these cell surface antigens and antibodies that bind to them are referred to in the present specification (see, for example, page 13, line 33 to page 14, line 4; page 15, lines 27-37; and page 24, lines 29-31). We submit, therefore, that it would be clear to a person skilled in the art that the MPCs defined in the present claims are characterised by expression of particular cell surface molecules such as STRO-1, VCAM-1, THY-1, etc. and that the claims as amended meet the requirements of 35 U.S.C. §112, second paragraph.

On page 3, in paragraph 6C of the office action, the Examiner rejected claim 58 for the reasons stated. In response, Applicants have amended claim 58 so that it is dependent on claim 53 rather than claim 25.

It is respectfully submitted with the presented of the instant amendment, the claims are now in compliance with the requirements of 35 U.S.C. §112, second paragraph.

The §112, First Paragraph Rejections

The New Matter Rejection

The Examiner has rejected claims 26, 52 and 56 under 35 U.S.C. §112, First Paragraph for the reasons which are set forth in the specification in paragraphs 7-8 of the present application. In response, Applicants provide support in the originally filed application for each of the passages in the claims which the Examiner contends constitutes new matter.

Alleged New Matter	Specification Reference
i) Claim 26: "surface markers specific for mesenchymal precursor cells consisting of integrin beta, STRO-2, CD146 or any combination thereof"	Integrin beta: A person skilled in the art would understand that integrin beta is an alternative name for CD29. Basis for this marker exists in the specification as filed at page 4, line 20 in conjunction with the reference to CD29 on page 6, line 31. STRO-2 and CD146: Figure 9 and page 13, line 4.
ii) Claim 52: "cells that are positive for one or more markers selected from the group consisting of $CD146^{bright}$ and $STRO\text{-}2^{bright}$"	Figure 9, two bottom dot plot histograms. The boxes represent cells that are both $STRO\text{-}1^{bright}$ and $CD146^{bright}$ (bottom left histogram) and $STRO\text{-}1^{bright}$ and $STRO\text{-}2^{bright}$ (bottom right histogram).
iii) Claim 56: "an enriched cell population as in claim 52 wherein the $CD146^{bright}$ cells carry a high copy number of an antigen identified by CD146	Figure 9, bottom left histogram - the boxed cells are those that are $CD146^{bright}$ which a person skilled in the art would understand are cells that have a high copy number of CD146.
iv) Claim 57: "an enriched cell population as in claim 52 wherein the $STRO\text{-}2^{bright}$ cells carry a high copy number of an antigen identified by STRO-2"	Figure 9, bottom right histogram - the boxed cells are those that are $STRO\text{-}2^{bright}$ which a person skilled in the art would understand are cells that have a high copy number of STRO-2.
v) Claim 58: "an enriched cell population as in claim 25 wherein $STRO\text{-}1^{bright}$ cells are negative for at least one marker selected from the group consisting of CBFA-1, collagen type II, PPARγ2, and glycophorin A"	Page 6, lines 36-37.

Thus, as presented above, each of the asserted phrases which the Examiner has contended constitutes new matter actually is completely supported by the originally filed specification. Consequently, the presently amended claims have not added new matter and the Examiner is respectfully requested to withdraw this rejection of the present application.

Lack of Enablement

On page 3, paragraph 9, the Examiner has rejected claims 22-35 31, 34, 40-45, 47-51 and 58 under 35 U.S.C. §112, first paragraph. The Examiner states that while the specification is enabling for an enriched cell population wherein at least 1% of the cells are mesenchymal precursor cells capable of giving rise to colony forming units - fibroblast (CFU-F), it does not reasonably provide enablement for an enriched cell population wherein at least 1% of ***any*** cells are capable of giving rise to CFU-F.

In response, Applicants have amended the claims to insert the limitation that the enriched cells are *mesenchymal precursor cells* capable of giving rise to CFU-F. Accordingly, Applicants respectfully submit that this amendment now renders the Examiner rejection moot.

The §102 Rejections

The Rejection over Simmons, et al.

On page 6, in paragraph 11, the Examiner rejects claims 25-28, 31, 34, 40, 41, 44, 48, 49 and 52-58 under 35 USC 102(b) as being anticipated by Simmons *et al* (presented in the IDS). Applicants respectfully traverse the Examiner's rejection in light of the amendment to the claims. As set forth in the amended claims, the invention relates to an enriched cell population wherein greater than 1% of the cells are mesenchymal precursor cells capable of giving rise to CFU-F. Accordingly, as amended, the claims clearly distinguish the present claims from the disclosure of Simmons *et al.*

The Simmons *et al* reference teaches that mesenchymal precursor cells can be enriched to some extent from freshly harvested bone marrow cells by selecting for cells that express the STRO-1 cell surface marker. As explained by Simmons *et al* at pages 272-273, it is known that bone marrow cells contain a proportion of multipotent stromal progenitors (referred to in the

present specification as mesenchymal precursor cells or MPCs) that are capable of giving rise to CFU-F. These CFU-F in turn are capable of giving rise under appropriate conditions to a broad spectrum of fully differentiated connective tissue, including cartilage, bone, adipose tissue, fibrous tissue and myelosupportive stroma.

As mentioned by Simmons *et al* in the paragraph bridging pages 272 and 273, MPCs and CFU-F are typically present at a very low incidence in bone marrow cells and this rarity was a major limitation to their study at that time. The present specification confirms this at page 3, lines 18-24 and states that the incidence of MPC in freshly isolated bone marrow cells is between 0.05%-0.001%. The important finding discussed in the Simmons *et al* citation is the identification that these MPCs could be enriched from freshly isolated bone marrow cells to some extent by selecting for STRO-1 positive cells. In particular, the selection of STRO-1 positive cells enabled isolation of MPCs (and resultant CFU-F) free of contaminating hemopoietic progenitors. The Simmons *et al* citation therefore represented a significant development in the technology at the time. Clearly, however, this citation is nothing more than a teaching that MPCs can be enriched to some extent from freshly isolated bone marrow cells by selection of STRO-1 positive cells (see the summary on page 278).

In contrast, the present invention represents an important advance over the Simmons *et al* disclosure because it is based on the identification of a subpopulation within this fraction of STRO-1 positive cells that contains MPCs. In particular, the present inventors sorted the STRO-1 positive cell population disclosed by Simmons *et al* into three subsets: STRO-1^{dull}, STRO-$1^{intermediate}$ and STRO-1^{bright}. Clonogenic assays for CFU-F in the different sorted subpopulations demonstrated that the vast majority of the MPCs are contained within the STRO-1^{bright} fraction (see page 23, lines 19-32).

The present inventors went on to further characterise this important subpopulation by showing that the STRO-1^{bright} subset containing the MPCs can also be identified by the VCAM-1^{bright} marker (see Figure 2a). Further studies showed that the incidence of clonogenic cells

within this subpopulation is one per two cells plated (see page 14, lines 35-37, page 24, lines 9-11 and Figure 2c). This represents an enriched cell population in which approximately 50% of the cells are MPCs that are capable of giving rise to CFU-F.

The inventors then went on to further characterise this subpopulation by probing for additional cell surface markers (see page 13, lines 1-4 and Figure 9). These results showed that the subpopulation of cells containing MPCs can also be identified by any one of the VCAM-1^{bright}, THY-1^{bright}, $CD146^{bright}$ or STRO-2^{bright} markers.

In summary, the present inventors have taken the STRO-1 positive fraction described by Simmons *et al* and have identified an important subpopulation within this fraction which contains the MPCs. Results presented in the present application show that this subpopulation can be identified by a number of different markers including VCAM-1^{bright}, THY-1^{bright}, $CD146^{bright}$ or STRO-2^{bright}. The identification of this subpopulation together with a description of markers that can be used for its isolation enables a substantially higher level of enrichment of MPCs than that achieved by the method described in the Simmons *et al* citation.

As the Examiner points out, the Simmons *et al* reference is silent about the percentage of MPCs capable of giving rise to CFU-F within the STRO-1 positive fraction of bone marrow cells. We submit, however, that evidence showing the inherent levels of MPCs present in the STRO-1 positive subpopulation described by Simmons *et al* is provided in Figure 9 of the present specification. As is clear from the legend for Figure 9 on page 13, this figure represents an analysis of STRO-1 positive human bone marrow cells (ie. the same population of cells described by Simmons *et al*). The results presented in Figure 9 show that the critical subpopulation of phenotypically defined cells identified by the present inventors as containing MPCs (i.e., the boxed population in each histogram of Figure 9) is never higher than 2%. More specifically, the results presented in Figure 9 provide evidence to show that phenotypically-defined MPCs are inherently present in a STRO-1 positive fraction of bone marrow cells at a level of between 1.5-2%. Therefore, if only 1 out of every 2 phenotypically defined-MPC gives

rise to a CFU-F, then the inherent number of MPC giving rise to CFU-F in an initial STRO-1 positive fraction is 0.75 - 1.0 %.

The markers and methods described in the present application provide for the first time enrichment of MPCs giving rise to CFU-F to a level of greater than 1%. Applicants respectfully submit, therefore, that the amended claims are clearly novel over the Simmons *et al* citation. Withdrawal of this rejection is respectfully requested of the Examiner.

The Rejection over US 6,087,113 to Caplan, et al. ("The '113 Patent")

On page 6, in paragraph 12 of the office action, the Examiner has rejected claims 25-28, 31, 34, 40, 41, 44, 47, 48, 49 and 52-58 under 35 USC 102(e) as being anticipated by the teachings of the '113 patent as is evidenced by Simmons *et al* and the disclosure of the instant specification on page 16, lines 20-30. Applicants respectfully submit that the amended claims are patentable over the '113 patent for the reasons which are set forth below.

The Examiner asserts that the '113 patent teaches how to obtain an enriched cell population containing up to 95% of MPCs. The Examiner also asserts that the enriched population carry the STRO-1 antigen and alleges that the properties of the MPCs defined in the present claims are inherent in the cell population described in the '113 patent.

We submit, however, that it is clear from the face of the '113 patent that the ***mesenchymal stem cell (MSC)*** cell population described therein is completely different to the ***MPC*** population described and claimed in the present application.

First, the MSCs of the '113 patent carry different cell surface markers compared to the MPCs of the present application. The Examiner states that the MSCs described in the '113 patent carry the STRO-1 antigen, but in fact this is not the case. The passage referred to by the Examiner (column 40, lines 21-35) merely states that the MSC population was probed through

the use of commercially available antibodies and STRO-1. Importantly, however, the results of this probing are disclosed in Table 5 at column 40, lines 50-64 and show that STRO-1 is a marker that is ***absent*** from the cell surface of the MSCs (see second column).

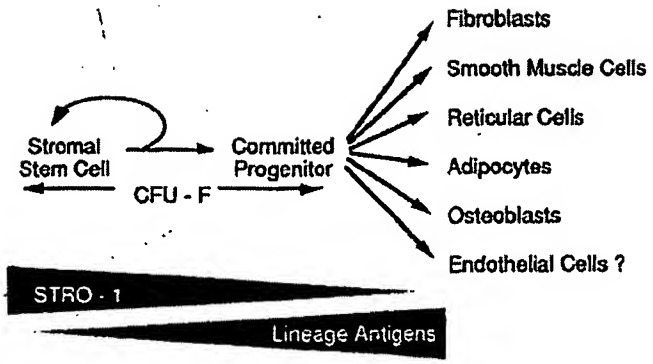
Further, the paragraph at column 40, line 65 to column 41, line 4 of the '113 patent states as follows:

> "Epitopes to markers that identify differentiated mesenchymal phenotypes are not detected by our analysis including those synthesized by chondrocytes (type II collagen, keratin sulfate (KS)), osteoblasts (Bone Gia Protein (BGP)), basement membrane fibroblasts (laminin, elastin and type IV collagen), marrow stromal cell progenitors (Stro-1 antigen), and endothelial cells (von Wilebrand factor)." [Emphasis added]

It is quite clear, therefore, that the MSC population described in the '113 patent is quite distinct from the MPC population of the present invention.

Importantly, the presently amended claims define an enriched population of cells wherein greater than 1% are MPCs capable of giving rise to CFU-F.

The Examiner asserts that Simmons *et al* teach that the ability to give rise to CFU-F is an inherent property of mesenchymal precursor cells. In fact, on page 273 Simmons *et al* teach that it is an inherent property of the ***stromal stem cell*** population to give rise to CFU-F. This is shown in Figure 1 of Simmons *et al* as follows:



As mentioned above, the '113 patent clearly states in the paragraph bridging columns 40 and 41 that the MSCs do ***not*** include markers synthesised by marrow stromal cell progenitors such as the STRO-1 antigen. This is consistent with the fact that the MSCs of the '113 patent were obtained following culture-expansion of bone marrow cells. See, for example, Example 4 of the '113 patent at column 39 which states as follows:

> "In a previous study, we reported on the isolation and culture-expansion of mesenchymal progenitor cells from human bone marrow with osteogenic and chondrogenic potential (Haynesworth, S. E. et al., *Bone* 13:81-88; 1992). We refer to these cells as Mesenchymal Stem Cells (MSCs)."

Simmons *et al* teach that expression of the STRO-1 marker decreases during development of stromal progenitors cells, which would occur during culture-expansion. It is therefore appropriate to conclude that the MSC population described in the '113 patent in fact represents MPC-derived cells at a later developmental stage and that this MSC population does not include marrow stromal cell progenitors that are capable of giving rise to CFU-F. Consequently, the '113 patent cannot be viewed as anticipating the present invention.

The Obviousness Rejections

On page 8, in paragraphs 13 and 14, the Examiner has rejected claims 25, 45 and 47 under 35 U.S.C. §103 as being obvious over Simmons, et al. in view of the '113 patent for the

reasons which have been detailed in the office action in paragraph 14 bridging pages 8 and 9. In paragraph 15 on page 9, the Examiner has rejected claims 25, 42, 43, 45, 50 and 51 as being obvious over Simmons, et al or the '113 patent in view of US patent no. 5,591,625 ("the '625 patent"). Applicants respectfully traverse the Examiner's rejection and assert that the newly presented claims are clearly patentable over the teachings of the prior art.

Applicants respectfully submit that the novelty arguments presented in the sections above also address the obviousness rejection set out in paragraphs 13-15 of the office action. Clearly, neither Simmons, et al. nor the '113 patent teach the instant invention, and the '625 patent does not in any way either teach or suggest the deficiencies of the Simmons, et al. and the '113 patent which fail to render the present invention obvious. It is respectfully submitted that the instantly claimed invention is non-obvious over the teachings of the cited references inasmuch as the presently claimed invention now clearly distinguishes over the teachings of Simmons, et al. and the '113 patent for the same reasons the present invention is novel over these teachings, and the '625 patent fails to obviate the deficiencies of Simmons, et al. and the '113 patent. In order to make out a cogent argument that the claims are obvious, one must posit a set of references which, *when combined*, teach the claimed invention. The cited references clearly do not. Consequently, it is respectfully submitted that the presently claimed invention is non-obvious over the teachings of the cited prior art.

The Provisional Obviousness-Type Double Patenting Rejection

The Examiner has provisionally rejected claims 25-28, 31, 34, 40, 41, 44, 48, 49 and 52-58 of the instant application under the judicially created doctrine of obviousness-type double patenting over claims 1-39 and 68-78 of copending application no. 10/813,747. Applicants respectfully submit that it is appropriate to address this issue in the '747 application and if applicable, to file a terminal disclaimer in the '747 application, rather than address that rejection here. For this reason, Applicants respectfully request that the Examiner withdraw this rejection in this application, so that this application can be allowed to issue.

For the reasons which are presented above, Applicants respectfully submit that the present application is in condition for allowance and such action indicating the allowability of the instant application is earnestly solicited. Applicants have amended a number of claims and have added no additional claims. Consequently, no fee is due for the presentation of the instant amendment.

If any fee is due for the presentation of the instant amendment, the Commissioner is hereby authorized to charge any fee due to deposit account 04-0838.

Respectfully submitted,
COLEMAN, SUDOL SAPONE, P.C.

By: _____
Henry D. Coleman
Reg. No. 32,559
714 Colorado Avenue
Bridgeport, Connecticut
(203) 366-3560

Dated: August 9, 2005

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